Plasma carotenoid response to chronic intake of selected foods and β-carotene supplements in men[1,2]

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ABSTRACT We determined serial changes in four major plasma carotenoid fractions (α-carotene, β-carotene, lutein/zeaxanthin, and lycopene) in 30 men consuming defined daily doses of carotenoids from foods (broccoli, carrots, or tomato juice) or from purified β-carotene in capsules (12 or 30 mg) for 6 wk while fed a controlled diet. Compared with baseline, β-carotene increased in the 30- and 12-mg-capsule and carrot groups whereas α-carotene increased in the carrot group and lutein increased in the broccoli group. Lower lutein concentrations in recipients of β-carotene capsules suggested an interaction between these two carotenoids. Lycopene declined in all groups except the tomato-juice group. Total carotenoid concentration changes only reflected the large increases in β-carotene concentrations and not the smaller changes observed in other individual carotenoids. Overall, purified β-carotene produced a greater plasma response than did similar quantities of carotenoids from foods sources. However, some foods increased plasma concentrations of certain carotenoids. *Am J Clin Nutr* 1992;55: 1120–5.

KEY WORDS β-carotene, carotenoids, foods, lycopene, lutein, α-carotene, supplements

Introduction

Epidemiologic studies on diet and cancer have indicated that consumption of carotenoid-containing foods may be associated with a reduced risk of certain epithelial cancers in humans (1–4), although the association of such foods (eg, cabbage, carrots) with prevention and/or treatment of cancer has been discussed since antiquity, beginning with the Roman, Cato the Elder (234–149 BC) (5). However, current attention to these foods has not been directed by history but has been a result of epidemiologic associations (6). Thus, the majority of chemotherapeutic and chemopreventive agents today are tested for efficacy in controlled clinical trials. Accordingly, numerous clinical trials are currently in progress to test whether chronic supplementation of purified β-carotene may prospectively reduce cancer rates. It is important to examine the plasma carotenoid response to β-carotene supplementation and to compare this response with that obtained with dietary intake of carotenoid-containing foods, especially those that have been associated with reduced risk of cancer (7).

Therefore, we determined the serial changes in seven plasma carotenoid fractions in 30 men given defined daily doses of carotenoids from carotenoid-containing foods (broccoli, carrots, tomato juice) or β-carotene from purified supplements (12 or 30 mg) for 6 wk on a controlled diet.

Subjects and methods

Thirty healthy men participated in a chronic carotenoid supplementation study conducted during October–December 1985. Volunteers were screened by using medical and dietary questionnaires, physical examination, hematologic and biochemical profiles, plasma carotenoid and retinol concentrations, and selected anthropometric measurements. Eligible subjects were between the ages of 20 and 45 y, had no history of chronic disease and no abnormalities on the basis of physical exams or blood profiles, were not smokers, were within 10% of ideal body weight for size, had baseline plasma total carotenoid concentrations between 1.3 and 3.7 μmol/L, and had no particular dietary patterns (eg, vegetarianism). Individuals not conforming to these eligibility criteria were excluded. Self-supplementation with vitamins was stopped by all subjects taking them ≥ 4 wk before the start of the study. Procedures used in the study were approved by committees for human subjects protection from the United States Department of Agriculture, the National Cancer Institute, and Georgetown University. Subjects were separated into two strata according to baseline total plasma carotenoid concentrations to identify subjects with concentrations above and below the mean. They were then randomly assigned to one of six treatment groups (five subjects per group) that were defined by the amount and type of total carotenoid supplementation received (**Table 1**).

Food analyses

All foods used as treatments were purchased in single lots before the study began in amounts sufficient to last for the entire treatment interval. Food samples were analyzed in the state of

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TABLE 1
Description of treatment groups by amount and type of carotenoid supplementation*

Treatment group	Carotenoid content				
	β-carotene	α-carotene	Lutein	Lycopene	Total carotenoids
			mg		
30 mg Carotenoids					
Purified β-carotene (n = 5; capsule)	30	ND	ND	ND	30
Carrots (n = 5; 272 g)	29	9	Trace	ND	38
12 mg Carotenoids					
Purified β-carotene (n = 5; capsule)	12	ND	ND	ND	12
Tomato juice (n = 5; 180 g)	Trace	ND	ND	12	12
6 mg Carotenoids					
Broccoli (n = 5; 300 g)	3	ND	3	ND	6
Placebo (n = 5; capsule)	ND	ND	ND	ND	ND

* ND, not detectable; trace, < 1 mg.

preparation (cooked) in which they were served to study participants. Multiple analyses of these samples of broccoli, carrots, and tomato juice were performed by using the methods of Khachik and Beecher (8) and Khachik et al (9). The quantities given to each group of subjects were determined on the basis of the chemical analyses of the vegetables and provided the desired daily carotenoid amount (Table 1).

Purified β-carotene source

The β-carotene capsules contained dry gelatin beadlets of 10% β-carotene compound with BHT, BHA, and sodium benzoate as preservatives (Hoffmann-LaRoche, Inc, Nutley, NJ). The placebo contained beadlets without β-carotene. Multiple analyses conducted in our laboratories confirmed the dose and composition of the synthetic capsules.

Controlled diets

Each participant consumed daily one of the six treatments at meals (split between lunch and dinner) together with a strictly controlled diet for 6 wk (42 d), followed by a 4-wk posttreatment period with a self-selected diet. The treatment foods were cooked according to instructions on the package from the supplier (eg, precooked, flash-frozen foods were boiled in serving bags). The controlled diet was formulated to have a constant and low total carotenoid content (0.5–1.6 mg/d for the 12 552-J intake amount), and to exclude carotenoid-containing foods used as treatments (eg, carrots, broccoli, and tomatoes). All meals were prepared under the supervision of a dietitian at the Human Studies Facility of the Beltsville Human Nutrition Research Center (BHNRC) at the US Department of Agriculture in Beltsville, MD. Breakfast, lunch, and supper were consumed in the dining facility on weekdays. All meals on weekends and holidays were prepared for carryout.

Seven days of menus were formulated from commonly available foods and were provided at 836-J increments between 10 878 and 16 736 J/d. Participants were started at estimated caloric intakes on the basis of stature and weight, and adjustments were made as needed to maintain constant weight throughout the study. All daily diets, regardless of the calories they provided, had fixed percentages of calories from fat (40%), carbohydrate (43%), and proteins (17%). Alcohol was not permitted during the controlled-diet period but instant coffee and diet lemon-lime, carbonated soft drinks were allowed ad libitum throughout.

Plasma analyses

Fasting blood samples were collected 1 wk before the start of the study, at baseline, and twice weekly during the 42-d, controlled-diet portion of the study for determination of plasma carotenoids, retinol, α-tocopherol, high-density-lipoprotein (HDL) cholesterol, low-density-lipoprotein (LDL) cholesterol, and triglyceride values. Blood collection continued at 7, 14, and 28 d posttreatment for all study participants.

Blood for carotenoid analyses was collected in all-plastic syringes, with 4500 u heparin/L whole blood. All samples were protected from light and were centrifuged at 2260 × g for 20 min at 12 °C within 1 h after blood drawing. Plasma samples were stored at −70 °C and were not thawed until analysis 17–19 mo later. Individual carotenoids and their total concentration were analyzed by HPLC methods of Bieri et al (10) as described by Craft et al (11). This analytical method measures 90% of the total plasma carotenoids present by summing the seven individual carotenoid peaks observed, including α-carotene, β-carotene, lutein/zeaxanthin, precryptoxanthin, cryptoxanthin, lycopene, and unidentified carotenoids. Crystalline α-carotene, β-carotene, lycopene (Sigma, St Louis), zeaxanthin, cryptoxanthin, and echinenone (Hoffmann-LaRoche, Inc, Nutley, NJ) were used as standards.

A pooled plasma reference material was analyzed daily in conjunction with all samples analyzed. Coefficients of variation for the seven carotenoid peaks that were summed and reported as total carotenoids varied from 2.8% (zeaxanthin/lutein) to 10.0% (precryptoxanthin).

Although the seven carotenoid fractions obtained were used to calculate total carotenoids, only the four major peaks (α-carotene, β-carotene, lycopene, and lutein/zeaxanthin) are reported in detail here. Triglycerides, cholesterol, and HDL cholesterol were analyzed on a Centrifichem Analyzer (Baker Instruments Corp, Allentown, PA) by using standardized procedures provided by the manufacturer. LDL cholesterol was estimated by the formula of Friedewald et al (12). Plasma retinol and α-tocopherol were determined by HPLC (13).

TABLE 2
Mean baseline concentrations of plasma carotenoids, and biochemical indices for study participants*

Carotenoids (mmol/L)	
β-carotene	303.1 ± 130.5
α-carotene	78.5 ± 53.7
Lutein/zeaxanthin	409.6 ± 131.1
Lycopene	892.3 ± 336.1
Total carotenoids	2139.0 ± 564.7
Biochemical indices	
Retinol (μmol/L)	2.31 ± 0.46
Tocopherol (μmol/L)	22.74 ± 5.49
Cholesterol (mmol/L)	4.09 ± 0.77
HDL cholesterol (mmol/L)	1.26 ± 0.22
LDL cholesterol (mmol/L)	2.53 ± 0.75
Triglycerides (mmol/L)	0.66 ± 0.22
Body mass index†	23.4 ± 1.6

* $\bar{x} \pm$ SD; $n = 30$.
† Weight (in kg)/stature^2 (in m^2).

Statistical analyses

Summary statistics were calculated for the carotenoids (response variables) for each of the six groups. The distributions of response variables were compared by using the *t* test for two independent groups, analysis of variance (ANOVA) for more than two independent groups, and nonparametric counterparts (the Wilcoxon two-sample rank-sum test and the Kruskal-Wallis test) (14). Comparisons of means were based on Duncan's multiple-range test as calculated with SAS (15). Paired *t* tests and the signed-rank tests were used to compare for significant changes above baseline.

Results

The 30 subjects entered into the study weighed between 64.1 and 88.2 kg and had baseline plasma total carotenoid concentrations that averaged 2139 ± 565 nmol/L. Baseline values of carotenoids and other biochemical indices at the start of the controlled-diet are shown in **Table 2.**

Results of plasma analyses for the four major carotenoids and for the total carotenoid concentration in each of the six groups

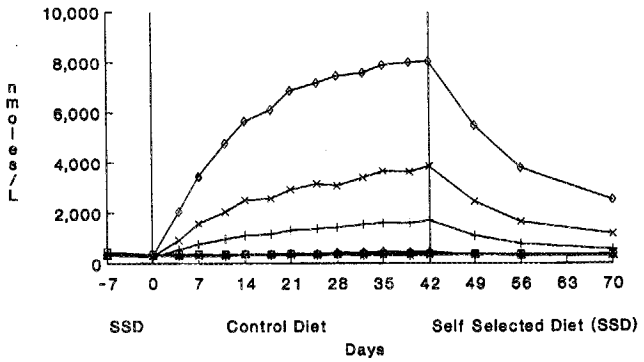

FIG 1. Mean plasma β-carotene response to foods and supplements. ◇, 30-mg β-carotene capsules; ×, 12-mg β-carotene capsules; +, carrots; △, broccoli; □, tomato juice; and *, placebo.

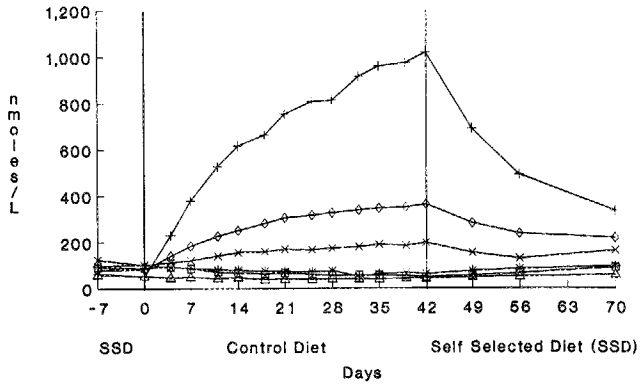

FIG 2. Mean plasma α-carotene response to foods and supplements. ◇, 30-mg β-carotene capsules; ×, 12-mg β-carotene capsules; +, carrots; △, broccoli; □, tomato juice; and *, placebo.

are shown in **Figures 1–5**. There were no dramatic changes in carotenoid concentrations during the week of self-selected diets before the start of the treatment period.

Table 3 shows the average maximum change in carotenoid concentrations over the treatment period. Beta-carotene concentrations increased significantly from baseline values with intakes of 30- and 12-mg capsules and with carrots. No significant increases in β-carotene concentrations were seen in the other treatment groups. The average maximum change was greatest in the 30-mg-capsule group, followed by the response of the 12-mg group and the carrot group. These three groups differed significantly from each other and all had a significantly higher average maximum change than did the placebo group.

Alpha-carotene concentrations increased to the greatest extent with carrots and the average maximum change was significantly different from all the other treatment groups. Although α-carotene in the β-carotene–supplement groups appeared to increase, only the 30-mg-capsule group had an average maximum change significantly different from that of the placebo group.

The average maximum change in lutein concentration in the carrot and tomato-juice groups did not differ from that of the placebo group. In all three groups the lutein concentration increased from baseline. However, the average maximum change in lutein concentration in the supplemented groups decreased

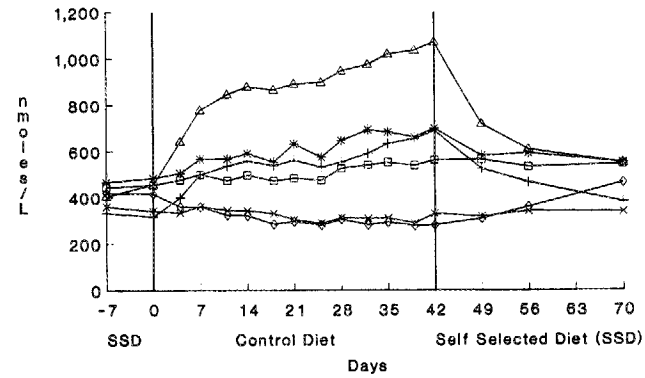

FIG 3. Mean plasma lutein response to foods and supplements. ◇, 30-mg β-carotene capsules; ×, 12-mg β-carotene capsules; +, carrots; △, broccoli; □, tomato juice; and *, placebo.

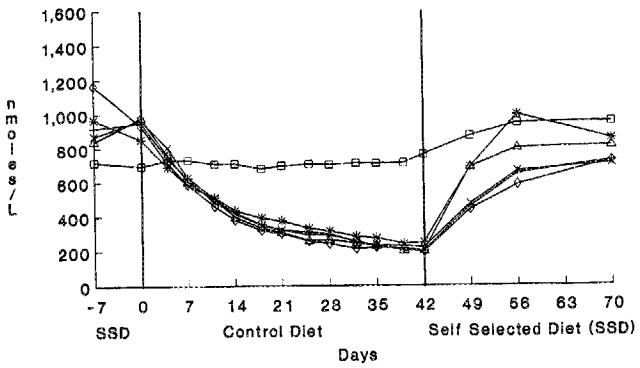

FIG 4. Mean plasma lycopene response to foods and supplements. ◇, 30-mg β-carotene capsules; ×, 12-mg β-carotene capsules; +, carrots; △, broccoli; □, tomato juice; and *, placebo.

from baseline and was significantly lower than that in the placebo group; the broccoli group had a significantly greater average maximum change than all groups.

During the treatment phase, plasma lycopene concentration declined consistently from baseline in all groups except the tomato-juice group. The tomato-juice group had a slightly positive average maximum change that was significantly different from all other groups.

The average maximum change in total carotenoid response was largely determined by increases in β-carotene concentrations in the β-carotene–supplemented and carrot groups. The average maximum change in total carotenoid concentration in the broccoli and tomato-juice groups did not differ from that in the placebo group.

No treatment group reached plasma steady state for β-carotene or total carotenoids insofar as plasma concentrations were still rising at 42 d and declined immediately after cessation of treatment, simulating a peak on the final day of treatment. The carotenoids that increased substantially during the treatment period decreased slowly during the 28 d of posttreatment observation. Some carotenoids, especially β-carotene in the capsule group and α-carotene in the carrot group, did not return to baseline values. Lycopene immediately increased after the treatment period in all groups except the tomato-juice group.

Figure 6 shows the mean plasma concentrations of carotenoids in the placebo group. Lycopene fell precipitously during the control-diet period and responded markedly after change to self-selected diets. In contrast, lutein increased whereas α and β-carotene remained relatively constant.

Discussion

When the study population was consuming self-selected diets, both plasma lycopene and lutein/zeaxanthin made up a greater percentage of the total carotenoids (43% and 19%, respectively) than did β-carotene (14%). Any suggested cancer-prevention agent in foods would, after consumption, probably be required to appear in the blood to a significant extent to have an effect on cancer sites other than those of the gastrointestinal tract (7).

We designed this study with a defined diet to control for dietary influences on absorption and utilization. Dimitrov et al (16) showed that dietary fat (63–69 g from breakfast and lunch plus an unreported quantity for the self-selected evening meal) affects the plasma response to β-carotene supplements. Our diet had 40% of the joules from fat, 17% from protein, and 43% from carbohydrates; it was moderately low in total carotenoids (0.5–1.6 mg/d at the energy intake of 12 552-J). Furthermore, the treatment doses were strictly monitored throughout the study to ensure compliance. The plasma samples were stored for 17–19 mo at −70 °C without thawing before analysis. Under identical conditions in a controlled storage study, we observed no significant deterioration in carotenoid concentrations for ≤ 28 mo (11).

Plasma β-carotene concentrations increased dramatically in response to purified β-carotene. Our data confirm earlier studies demonstrating a substantial increase in serum or plasma β-carotene in response to purified β-carotene at concentrations as low as 12 mg for 42 d (16, 17). Previously we reported carotenodermia in subjects ingesting the 30-mg capsules during this study (18). Constantino (17) noted a dramatic increase in β-carotene concentration after 2 mo of 15 mg β-carotene/d followed by a plateau in concentration after 4 mo. Plasma β-carotene concentration in our supplemented groups (30 and 12 mg β-carotene/d) also showed a sharp initial increase and may have stabilized if the supplementation had continued for a longer time.

Although the large serving (272 g) of carrots provided 29 mg β-carotene/d, the average maximum change in plasma concentration was only 18% of that in the group that received 30-mg capsules of β-carotene. Previous investigators noted that carotenoids in foods are much less available than are purified sources of β-carotene (19, 20).

It is interesting to observe that α-carotene concentrations increased in the purified β-carotene groups whereas α-carotene concentrations did not increase in the placebo group. This increase could not be due to the trace amount of α-carotene found in the purified β-carotene by chemical analyses. We speculate that this may be an analytical artifact because α- and β-carotene are not completely resolved to baseline concentrations when plasma contains high concentrations of β-carotene and as a result values for α-carotene may be increased.

Carrots provided a good source of both α- and β-carotene. Carotenes from cooked carrots are absorbed intestinally more efficiently than are those from raw carrots because the plant cells are disrupted during preparation and because the mechanical homogenization of carrots allows greater efficiency of absorption

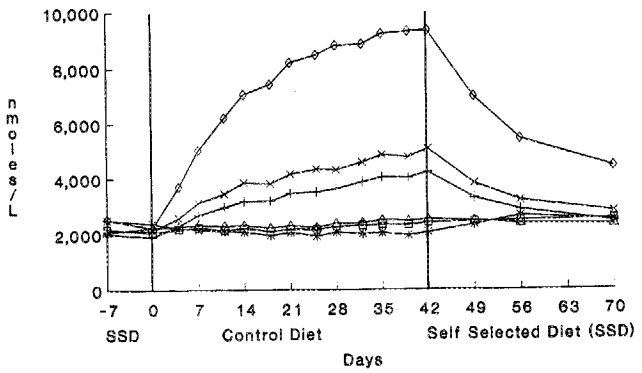

FIG 5. Mean plasma total carotenoids response to foods and supplements. ◇, 30-mg β-carotene capsules; ×, 12-mg β-carotene capsules; +, carrots; △, broccoli; □, tomato juice; and *, placebo.

(21). Alpha-carotene concentrations increased (in nmol/L per mg carotenoid) twice as much as those of β-carotene when the maximum change from the dose given was compared. These response differences may result from the more efficient conversion of β-carotene than of α-carotene to retinol (22). Alternatively, the absorption of the α-carotene may have been greater than the absorption of β-carotene from the same food source. Kim et al (23) also observed a greater serum-dose response for α-carotene than for β-carotene in subjects receiving carrot juice.

Lutein concentrations in the broccoli group increased significantly over time, reflecting the 3 mg lutein/d derived from broccoli; the maximum change of 679 nmol/L was significantly greater than the increase in the placebo group. In contrast, the maximum change in plasma β-carotene from broccoli did not differ significantly from that in the placebo group.

Plasma lutein concentrations increased when the placebo and the controlled diets were fed (Fig 6). However, when large daily doses of purified β-carotene were given (12 or 30 mg), the plasma lutein response was significantly lower than the response to placebo. Large doses of purified β-carotene may impair intestinal absorption of lutein. This suggestion of carotenoid interactions should be considered when the longer-term effects of β-carotene supplementation are evaluated.

Baseline lycopene concentrations in this predominantly college-aged study population were more than double those reported in a population of middle-aged men (24). The rapid decrease during the treatment period and increase afterwards for all groups except the tomato-juice group indicates that the self-selected diet of our subjects contained substantially more lycopene than did the controlled diet. Because tomatoes are the major contributor of dietary lycopene compared with other foods in the US diet (7), these observations suggest high intakes of tomatoes and/or tomato-containing foods. We are currently examining the food histories of our subjects to determine the types of food eaten and carotenoid intakes. Based on the lycopene concentration at day 0, the calculated half-life for all groups except the tomato-juice group was 11–14 d. When the natural logs of the lycopene concentration of these groups were plotted against time, only the first 14 d of decline were linear, indicating the existence of more than one body pool.

Although the changes in total carotenoid concentrations reflected the dramatic increases in β-carotene concentrations, more subtle changes in other carotenoids were not readily apparent. Significant changes in carotenoid concentrations in the broccoli- and tomato-treatment groups did not result in total carotenoid concentrations that were significantly different than those in the placebo group. These results emphasize the need for sensitive methods such as HPLC to detect alterations in individual carotenoid.

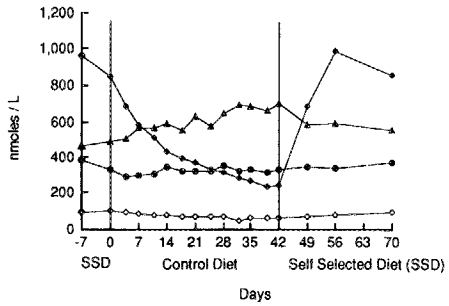

FIG 6. Mean response of plasma carotenoids to placebo capsule. ●, β-carotene; ◇, α-carotene; ▲, lutein; and ♦, lycopene.

Although the controlled diet was moderately low in calculated carotenoid content, the placebo group did not show any dramatic changes over the treatment period except for lycopene. This suggests that plasma carotenoid concentrations other than lycopene remain fairly constant and are slow to change with low dietary amounts. The gradual return toward baseline after supplementation for β-carotene in the capsule and carrot groups and in α-carotene in the carrot group indicates a slow turnover time for these carotenoids. For the period after supplementation we calculated a half-life of 7–14 d for β-carotene in the capsule and carrot groups and in α-carotene in the carrot group. When the natural logs of the carotenoid concentrations were plotted against time, each of the four graphs was linear during the first 14 d followed by a break or change in slope. This nonlinear decline indicates the existence of more than one body pool, similar to our observations for lycopene.

There appeared to be less variability in the plasma response to the continual supplementation of dietary carotenoids in this study population than there was in a different study population in our controlled study of a single dose of dietary carotenoids (25). With continual carotenoid supplementation, all participants in a treatment group showed plasma carotenoid responses in a continuum with other members of that group.

Despite dramatic increases in β-carotene in some groups, there were no significant changes in retinol concentration over time in any of the treatment groups. These data confirm observations

TABLE 3
Average maximum change in plasma concentrations of carotenoids, by type of supplementation*

Treatment group	β-carotene	α-carotene	Lutein	Lycopene	Total carotenoids
			nmol/L		
30 mg β-carotene (n = 5)	7901 ± 1381[a]	288 ± 75[a]	−152 ± 48[a]	−743 ± 565[a]	7344 ± 1781[a]
12 mg β-carotene (n = 5)	3589 ± 707[b]	98 ± 46[a,b]	−22 ± 105[a,b]	−756 ± 217[a]	2977 ± 647[b]
Carrots (n = 5)	1438 ± 762[c]	971 ± 377[c]	381 ± 257[c]	−747 ± 247[a]	2366 ± 1542[b]
Broccoli (n = 5)	193 ± 132[d]	−13 ± 31[b]	679 ± 151[d]	−778 ± 215[a]	302 ± 465[c]
Tomato juice (n = 5)	17 ± 93[d]	−36 ± 40[b]	90 ± 107[b,c]	34 ± 306[b]	237 ± 567[c]
Placebo (n = 5)	21 ± 111[d]	−57 ± 54[d]	233 ± 199[c,e]	−612 ± 234[a]	−469 ± 501[c]

* $\bar{x}$ ± SD. Means in the same column with different superscripts are significantly different, $P < 0.05$.

by others that circulating retinol does not appear to be affected by long-term doses of purified β-carotene in subjects with normal baseline concentrations (26).

Overall, the plasma response to purified β-carotene was greater than it was to similar quantities of carotenoids from food. These micronutrient supplements appear to be more readily absorbed than are the same micronutrients in foods, in terms of producing a plasma response. We did not measure metabolic indices other than plasma response, however. Given the marginal plasma responses observed to some foods, it may be difficult to markedly raise the blood concentrations of some carotenoids when common foods are the sole source. Participants in the present study attested that it would be difficult to physically consume daily amounts of carrots (272 g) or broccoli (300 g) much greater than the amounts provided in the study diets. Levels of consumption of tomato juice (180 g/d), however, could have been markedly increased without gastric discomfort. Consumption of tomato juice at amounts up to 2 L/d for several years have been reported, resulting in lycopenemia (27). This level of consumption is more than 10-fold that in the present study. In addition, among the general population, some individuals may have low carotenoid concentrations irrespective of diet. Differences in absorption, transport, and efficiency in retinol conversion may affect plasma carotenoid concentration.

To achieve major increases in specific plasma carotenoid concentrations, it may be more effective to ingest purified carotenoids than to attempt major alterations in the human diet. However, foods high in carotenoids can contribute smaller but significant increases in certain carotenoids and also provide many other biologically active constituents that are not present in purified micronutrient supplements. These constituents also may be important for decreasing the risk of cancer and/or contributing to optimal health (7).

Better understanding of carotenoid metabolism and the bioavailability of carotenoids from foods prepared in different ways will be important to formulating meaningful recommendations regarding dietary carotenoid consumption.

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